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Supplementary notes on *Basidiopycnis hyalina* (*Basidiomycota, Atractiellales*) and its anamorph

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Abstract — Publications concerning the auricularioid and pycnidial basidiomycete *Basidiopycnis hyalina* and its anamorph are reviewed. The hyphomycetous anamorph of *Basidiopycnis hyalina* is considered identical with the recently proposed taxon *Basidiopycnides albertensis*. Study of a type specimen potentially related to this anamorph revealed a new synonym, *Botryonipha dubia* (≡ *Stilbella dubia*, ≡ *Stilbum dubium*) for the discomycete *Neodasyscypha cerina*. *Basidiopycnis* and its anamorph are excluded from the coelomycetes. Complementary data are presented for the phylogenetic relationship between the coelomycetous genus *Chaetospermum* and the teleomorphic genus *Efibulobasidium* of the *Sebacinales*. Re-examination of another recently described pycnidial basidiomycete with auricularioid basidia, *Mycogelidium sinense*, revealed that conidiophores of a coelomycetous sooty mould with relationship to the *Capnodiales* had been mistaken for basidia.

Key words — *Conidiocarpus*, *Helotiales*, sooty moulds, ultrastructure

Introduction

Basidiopycnis hyalina Oberw. et al. is a minute basidiomycete with auricularioid basidia developing within a white, conical peridium. The anamorph is hyaline, synnematous and has annellidic conidiogenous cells and one-celled conidia. This fungus was found in bark beetle galleries and was described as a new genus and species by Oberwinkler et al. (2006). The passively released basidiospores and the slimy masses of conidia were considered adaptations for dispersal by bark beetles. Subsequently, three other publications appeared that relate in various ways to the description of *B. hyalina*. Hausner et al. (2008) treated the anamorph, Rungjindamai et al. (2008) treated the anamorph and teleomorph, and Zhuang & He (2007) described a new species, genus, and family of

teleomorphic basidiomycetes with auricularioid basidia formed within peridia. In our opinion, some of the results and conclusions in these three publications are incorrect or deserve more detailed analysis. The present paper discusses these issues and contains complementary notes to our publication (Oberwinkler et al. 2006). The present note presents new data and alternative interpretations of previously published data.

Basidiopycnis hyalina

In our description of *B. hyalina*, we did not propose a formal name for the anamorph stage because it can be connected with the teleomorph beyond any doubt. The available data was insufficient for the morphological distinction between this anamorph and similar anamorphs (for examples see Oberwinkler et al. 2006). Finally, for basidiomycetous anamorphs with known teleomorph connections, there is a long tradition of not naming the anamorph stages separately, exemplified by the pioneers Brefeld (1888) and Buller (1924), and predominantly maintained in recent times in papers such as Botha & Eicker (1991), Frieders & McLaughlin (2001), Scheuer et al. (2008), Walther & Weiß (2006).

Another reason for not adding a second formal name to a single species was that the identity of several species mainly described in the 19th and beginning 20th century in *Botryonipha* Preuss, *Graphium* Corda, *Stilbella* Lindau, and *Stilbum* Tode has not been clarified, for example because type specimens could not be accurately reassigned or were not found (Seifert 1985). One of these doubtful *Stilbum* species, *Stilbum erythrinae* Hansf., was recently provisionally transferred to *Chionosphaera* D.E. Cox after study of the type (Kirschner & Chen 2008). For some of the other species, e.g. *Graphium claviforme* ("*clavaeforme*") Preuss, Seifert (1985) mentioned a similarity with the anamorph of *Stilbotulasnella* Oberw. & Bandoni, which is presently morphologically indistinguishable from the anamorph of *Basidiopycnis hyalina* at the generic level (Oberwinkler et al. 2006).

In our attempts to trace back some of the unclear species of Seifert (1985), *Botryonipha dubia* was re-examined and excluded as a possible name for the anamorph of *Basidiopycnis hyalina*. The type specimen of *Graphium claviforme* was misplaced in B and could not be located and studied.

Neodasyscypha cerina (Pers. : Fr.) Spooner, in Suková, Czech Mycol. 57: 168, 2005

= *Botryonipha dubia* Preuss, Linnaea 25: 741, 1852; holotype, Preuss 624 (= 548 b), B 70 0014109!, **syn. nov.**

≡ *Stilbum dubium* (Preuss) Sacc., Syll. Fung. 4: 575, 1886

≡ *Stilbella dubia* (Preuss) Lindau, Nat. Pflanzenfam. (Leipzig) 1(1**): 489, 1900

For further synonyms see Suková (2005).

COMMENTS: Several ascomycetes were found in the type specimen of *Botryonipha dubia* but the morphology of only one species conformed to the description of Preuss (1852). This species was represented by young stalked ascomata, with the apex densely covered with olive-yellowish hairs when seen with the dissecting microscope. In the light microscope, the hairs appeared brown, septate, thick-walled, and densely covered with warts of different sizes, which are considered the diagnostic characteristics for the genus *Neodasyscypha* Suková & Spooner (Suková 2005). Asci (approx. 50 × 4 µm) with a blue apical apparatus in Melzer's reagent and ellipsoidal to slightly fusoid ascospores (5–6 × 2 µm) also supported the identification of *B. dubia* as young ascomata of *N. cerina*. The warts were apparently mistaken by Preuss (1852) for conidia and the hairs for conidiophores ("fibrillis septatis, longis, sporidiferis"). This new heterotypic synonym and its corresponding homotypic synonyms are additions to the curious history of nomenclature of this species (Suková 2005).

Hausner et al. (2008) made an important contribution to the knowledge of the anamorph of *Basidiopycnis hyalina* with their first published record from America. However, because they did not find the teleomorph, the species could be identified only in its anamorph by a combination of light microscopic and molecular sequence comparisons. The light microscopic characteristics were identical to those presented by Oberwinkler et al. (2006). The authors emphasized the spaces between the annellations of the conidiogenous cell visible by light microscopy, which are described differently by Hausner et al. (2008) and Oberwinkler et al. (2006). The minor differences can be explained easily. Fresh material was used for characterizing the annellidic conidiogenous cells in Hausner et al. (2008), but Oberwinkler et al. (2006) employed dried specimens. For illustrating the general identity of the morphology of conidiogenous cells, we reproduce here (FIG. 1) illustrations based on fresh material from Kirschner (1994). In Hausner et al. (2008), the ultrastructural differences between the anamorph of *B. hyalina* and similar anamorphs were taken from the literature. The status of previously described species of uncertain identity, mentioned above, was not addressed by Hausner et al. (2008).

Although no new data for distinguishing the anamorph of *B. hyalina* from similar anamorphs were presented by Hausner et al. (2008), they formally proposed a new genus and species, *Basidiopycnides albertensis*, for the anamorph of *B. hyalina*. The only differences between the American and European strains found by these authors were 3 substitutions in the 18S rDNA sequence and 16 substitutions in the complete ITS sequence. Because the ITS sequences compared are 405 bp long, this makes a 3.9% overall difference. For the American strains, five isolates from the same locality were used, two originating from the same substrate and therefore possibly genetically identical. As shown by Nilsson et al. (2008), intraspecific variability of ITS sequences

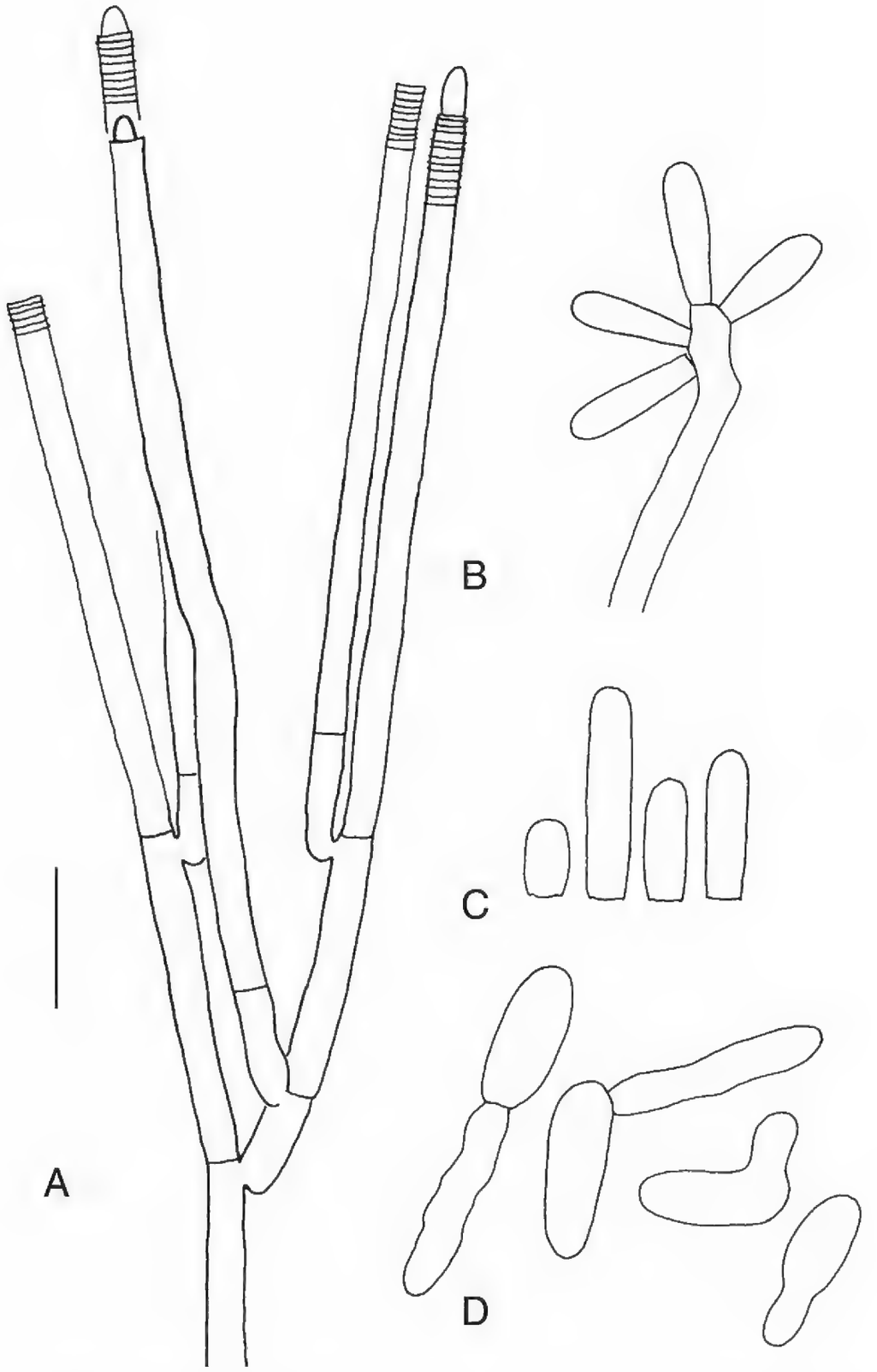

Fig. 1. *Basidiopycnis hyalina*, anamorph from living culture (reproduced from Kirschner 1994). Scale bar = 10 µm. A. Conidiophore with individual annellations. B. Apex of an atypical conidiogenous cell from nutrient-rich medium with conidia still attached. C. Conidia. D. Germinating conidia.

can vary between 0 to 17.3%, and therefore the 3% threshold applied by some authors cannot be generalized for all fungal species. The systematic significance of different ITS positions within morphologically identical specimens can, therefore, be evaluated only when a significant number of strains from different geographical areas are compared. Since this is not the case for *B. hyalina* and its anamorph, we consider that the specimens from Europe and America represent a single species, with *Basidiopycnis hyalina* as teleomorph and *Basidiopycnides albertensis* as its anamorph. Studies of hitherto not clarified type specimens and of similar anamorphs are still necessary for adequate morphological characterization of the anamorph. More strains from other regions than those presently available will be required for reliable molecular analyses of species delimitations.

Basidiomycetous, but not all coelomycetous

Rungjindamai et al. (2008), in treating several anamorphic *Basidiomycota* with a molecular approach, showed phylogenetic relationships between certain coelomycetes and sexual basidiomycetes. The authors, however, confused hyphomycetes, coelomycetes, and basidiomycetes. *Basidiopycnis hyalina* ("*Basidiopycnis hyaline*"), *Helicogloea angustispora* L.S. Olive, and *Leucogloea compressa* (Ellis & Everh.) R. Kirschner are listed among "basidiomycete genera... with coelomycetous anamorphs" in the text and a table. The anamorphs of *B. hyalina* and *Helicogloea angustispora* and the anamorph species *L. compressa* are hyphomycetes, however, not coelomycetes. We admit that our naming of "*Basidiopycnis*" among "pycnidial members of the *Atractiellales*" (Oberwinkler et al. 2006) might be responsible for the connection of the teleomorph to the coelomycetes by Rungjindamai et al. (2008). Circumscribing this teleomorph genus and species as "gasteromycete", i.e. in a purely morphological sense referring to basidia developing within a peridium, would avoid an affiliation with anamorphic coelomycetes more effectively than using the term "pycnidial members". In the list of coelomycetes with relationships to basidiomycetes in Rungjindamai et al. (2008), *Leucogloea compressa* is given as teleomorph of *Pleurocolla compressa* (Ellis & Everh.) Diehl, but in fact both names are homotypic synonyms of the same anamorph. Furthermore, the pycnidial *Ditangium* P. Karst. anamorphs of *Craterocolla* Bref. species, also belonging to the *Sebacinales* (Brefeld 1888, Weiß et al. 2004), were not listed.

Rungjindamai et al. (2008) showed a phylogenetic relationship between species of the coelomycete genus *Chaetospermum* Sacc. and the *Sebacinales*. A connection between *Chaetospermum* and a known teleomorphic genus of the *Sebacinales* could not be suggested. Unfortunately, the authors were unaware of the note by Wells & Bandoni (2001), who recorded a *Chaetospermum* state in cultures of *Efibulobasidium albescens* (Sacc. & Malbr.) K. Wells. We can

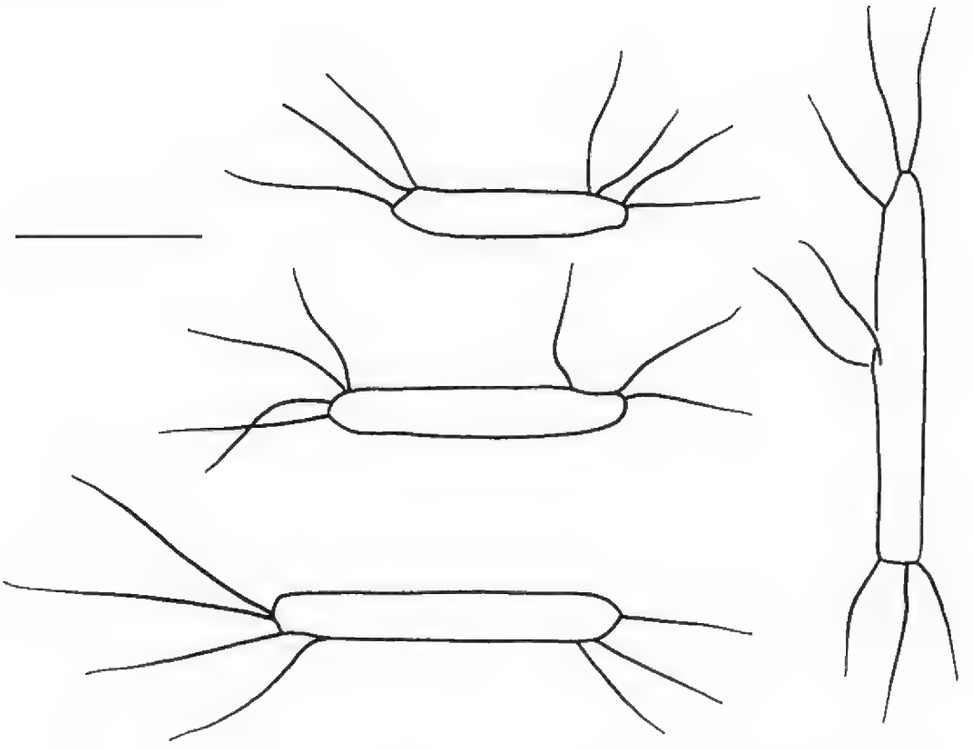

FIG. 2. Conidia of *Chaetospermum gossypinum* derived from cultivating *Efibulobasidium albescens* (R. Kirschner & C.-J. Chen 1316, TNM). Scale bar = 20 µm.

indirectly support this connection of *Chaetospermum* with the *Sebacinales* first shown by Wells & Bandoni (2001) with observations on *Ch. chaetosporum* (Pat.) A.L. Sm. & Ramsb. and *E. albescens*. Both are the type species of their respective genera, but were not included in the analyses by Rungjindamai et al. (2008). Our observations were based on material collected in Taiwan: *Chaetospermum chaetosporum*, on dead herbaceous stem, Taiwan, Hsinchu County, between Chutong and Guanwu Park, ca. 1,250 m, 3 Mar 2002, R. Kirschner & C.-J. Chen 1097; *Efibulobasidium albescens*, on dead twig, Taiwan, Chiayi County, Alishan, ca. 1,500 m, 8 June 2002, R. Kirschner & C.-J. Chen 1316 (TNM), a living culture could not be preserved, but a permanent slide of conidia from culture was saved (TNM). On the specimen of *E. albescens*, we found basidiospores of *E. albescens* together with conidia of *Chaetospermum gossypinum* (G.F. Atk.) Nag Raj in the same fructifications. In a pure culture of *Ch. gossypinum* derived from this material (R. Kirschner & C.-J. Chen 1316), conidia measured (24–) 25.5–37.5(–46) × 4–5(–6) µm and had 3–4 polar and subpolar filamentous appendages, in some cases with additional, more lateral appendages (FIG. 2). Furthermore, we found dolipore septa with continuous parenthesomes typical of *Sebacinales* (and only few other basidiomycetous lineages) in *Ch. chaetosporum* collected in the field (R. Kirschner & C.-J. Chen 1097; FIG. 3). Identification of *Chaetospermum* species without re-examination of types remains tentative according to Nag Raj (1993). More detailed investigation of the morphology of

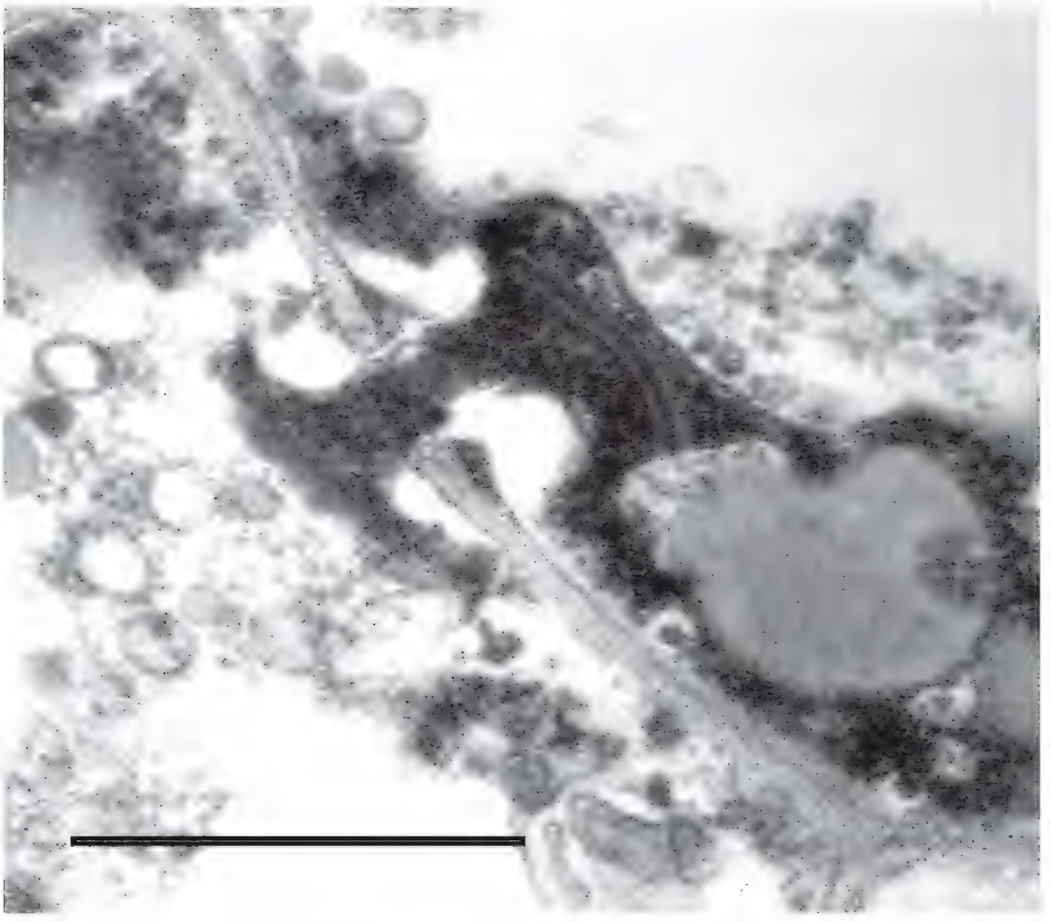

FIG. 3. Ultrastructure of hyphal septum of *Chaetospermum chaetosporum* (R. Kirschner & C.-J. Chen 1097, TNM) showing a dolipore with a continuous parenthesome (the other parenthesome hardly visible probably due to bad fixation). Scale bar = 0.5 μm.

Efibulobasidium species in culture and molecular analyses including the type species of *Chaetospermum* and *Efibulobasidium* will contribute to a clarification of the anamorph-teleomorph relationship.

Coelomycetous, but not basidiomycetous

Zhuang & He (2007) published a new family, *Mycogelidiaceae* W.Y. Zhuang based on a new genus and species from China, *Mycogelidium sinense* W.Y. Zhuang & X.S. He. Pigmented pycnidia are formed on large, strongly branched coralloid thalli, several cm broad. Four-celled, transversally septate hyphae giving rise to spores on peg-like outgrowths in the pycnidia were interpreted as auricularioid basidia. The illustrations of the fructification composed of pycnidia partly embedded in branched, sterile stalks, however, indicate a relationship to coelomycetous sooty mould genera such as *Conidiocarpus* Woron., *Conidioxyphium* Bat. & Cif., and *Podoxyphium* Speg., which are pycnidial anamorphs of *Capnodiales*, *Ascomycota* (Batista & Ciferri 1963, Hughes 1976). These anamorphs are reported to attain dimensions of several

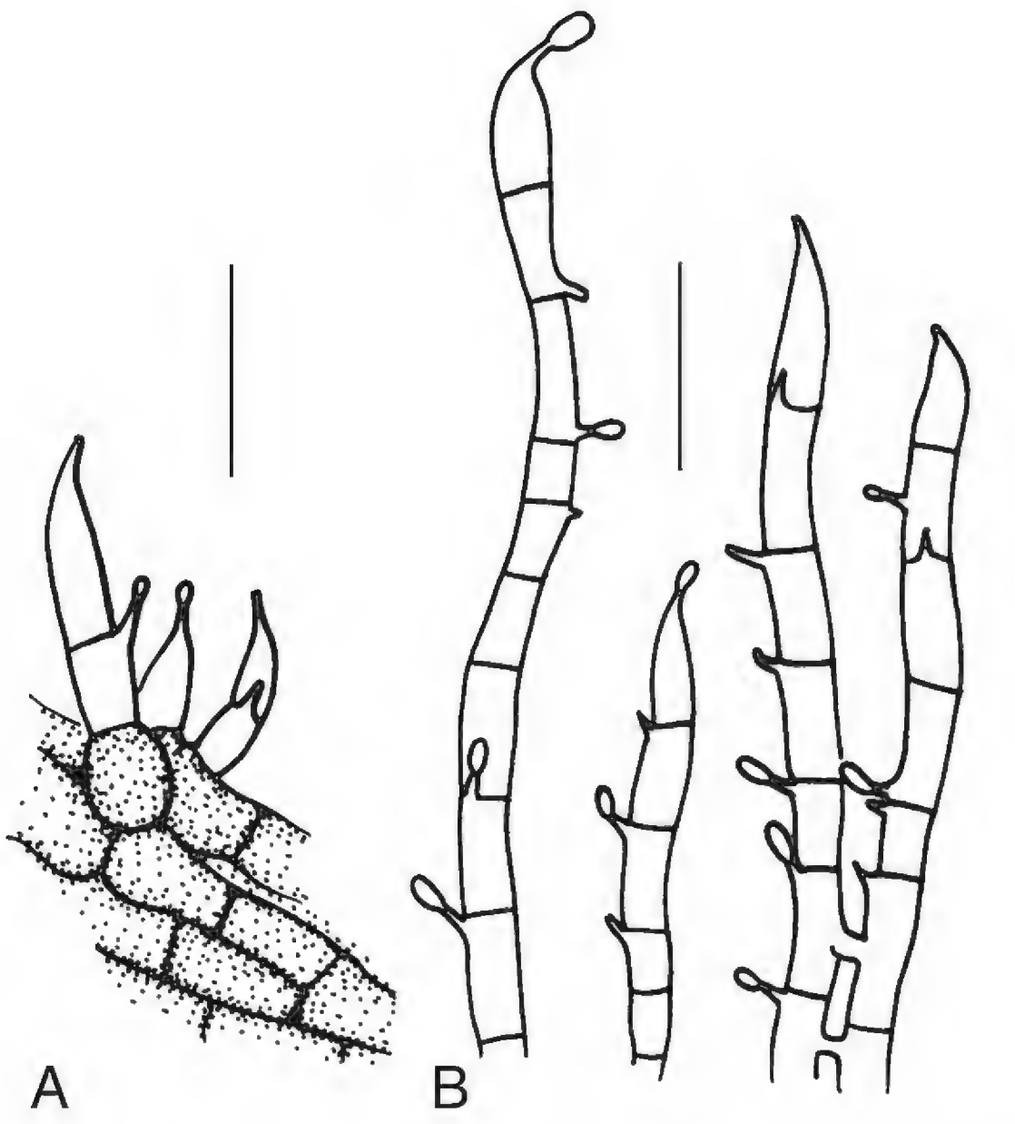

Fig. 4. *Mycogelidium sinense*, conidiophores from isotype (HMAS 97522), squash mount in 5% KOH and 1% aqueous phloxine, observed with phase contrast. A. One- and two-celled conidiophores arising from the pycnidial wall. B. Several-celled conidiophores. Scale bar = 10 µm.

mm (Batista & Ciferri 1963), which are even exceeded by the Chinese specimens. Re-examination of the isotype of *M. sinense* (HMAS 97522) revealed that the dark pigmented pycnidia often show a slight swelling above the terminal sterile, pale brown branch. The original description of auricularioid basidia was based on a misinterpretation of conidiophores with intercalary conidiogenous cells. We found 1- to 9-celled conidiophores directly arising from the pale brown inner wall of the venter-like pycnidial cavity, 4–46 × 2–3 µm, with individual conidiogenous cells, 3–8 × 2–3 µm, and a terminal, subterminal, basal, rarely lateral conidiogenous outgrowth per cell, 1–2 × 0.5 µm (Fig. 4). The apical point of these outgrowths was too minute to resolve any periclinal thickening or

annellidic wall structures with the light microscope. The family *Mycogelidiaceae* is considered an anamorphic family name corresponding to the *Capnodiaceae*. The habit of conidiomata and micromorphology of conidiophores and conidia indicate a relationship of *Mycogelidium sinense* to *Conidioxyphium* in the sense of Sutton (1980). *Conidioxyphium* and *Podoxyphium* were considered synonyms of *Conidiocarpus* by Hughes (1976). *Mycogelidium* is probably a synonym of *Conidiocarpus* in the sense of Hughes (1976). The invalid family "*Asbolisiaceae* Speg. char. em. Bat. & Ciff. (1963)" comprising capnodiaceous pycnidial fungi (Hughes 1976) corresponds to the *Mycogelidiaceae*. The pycnidial fungi mentioned here are generally connected to sexual *Capnodiaceae*, but details have not been sufficiently clarified (Hughes 1976). Though the generic delimitations of coelomycetous sooty moulds connected to the *Capnodiaceae* and related families were examined by Hughes (1976) and Sutton (1980), delimitations particularly at the species level are still very difficult and need a revision based on type studies, cultivation, and molecular approaches. At the present time, we cannot draw any taxonomical conclusions on *Mycogelidium sinense* and do not preclude eventual recognition of this taxon as a separate species or even genus, especially because of the unusually large fructifications.

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